Nota Lepi. **47** 2024: 49–56 | DOI 10.3897/nl.47.109920 Research Article

Rondotia melanoleuca sp. nov., a new wild-mulberry silkworm from China (Lepidoptera, Bombycidae)

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https://zoobank.org/3B0A899F-DCCB-4621-8F2F-C8346B740EF0

Received 22 July 2023; accepted 22 February 2024; published: 7 March 2024
Subject Editor: Rodolphe Rougerie.

Abstract. Several yellow larvae with black spots were discovered in the wild of Chinese Sichuan and Yunnan provinces, and were further raised in captivity. Reared adults exhibit a striking black and white wing pattern, and they represent unequivocally a new species, here described as *Rondotia melanoleuca* **sp. nov.** Molecular analyses suggest that this species could be sister to all previously known species of *Rondotia*.

Introduction

The genus *Rondotia* Moore, 1885 has long been identified as containing pests for the silk industry. Moore (1885) named this taxon in honor of the sericulturist Natalis Rondot, based on the type species *Rondotia menciana* Moore, 1885 from the Far East. For economic reasons, the biological characteristics and genomics of this white mulberry caterpillar have been studied in detail in China (e.g., Xu et al. 1994; Bai et al. 2001; Tang et al. 2018), while the other two members of the genus have received less attention. After the original description by Hampson (1893: 33–34), *Rondotia diaphana* (Hampson, 1893) from Myanmar, the species was later redescribed by Conte (1911: 72), and subsequently been reported from China (Wang et al. 2009), India, Thailand, and Vietnam (Zolotuhin and Witt 2009). In contrast, *Rondotia lineata* Leech, 1898 is a highland flyer endemic to the eastern edge of the Qinghai-Tibet Plateau and has rarely been mentioned in literature (Wang et al. 2015). Leech (1898) established the Korean *Rondotia lurida* Fixsen, 1887 as a synonym of *R. menciana*, and Conte (1911: pls. 5, 8) illustrated the three accepted taxa in colour. A recent taxonomic review shows the three species are closely related and monophyletic; macroscopically, these moths have similar wing patterns and venation (e.g., Wang et al. 2015).

We describe here a new species whose larvae were initially unidentified and collected in the wild in the Sichuan and Yunnan Provinces of China. Further morphological comparison and molecular data reveal its phylogenetic relationship within the genus *Rondotia*. It is currently only known from mid-elevation localities in southwest China.

Material and methods

A vernier caliper INSIZE 1108-150C 0–150 mm / 0.01 mm (± 0.02 mm) and magnifying lens PEAK 2008-50X 0–1.6 mm / 0.02 mm were used for measuring the dimensions of specimens. Genital preparations were made with 10% KOH solution (95 °C, 15 mins), and mounted the specimens on slides for observation and photography. Most of the color images were photographed with a NIKON D5500 and LAOWA 60 mm *f*/2.8–22 lens, but Fig. 1E–H were taken with a SONY α7 IV and objective lens 4× / 0.1. The images were focus stacked in PHOTOSHOP BETA, while visual optimization and stitching of figures was done with AFFINITY PHOTO 2.0.4.

A partial COI sequence (1235 bp) was newly sequenced from a paratype [unique identifier A23072707] of the new species, and the data has been submitted to NCBI [Genbank: OR051027]. We used the kit TIANGEN DP304 for DNA extraction (cryogenic grinding + silicon matrix adsorption) and PCR was conducted on a EASTWIN EDC810 thermocycler using 2× AceTaq Master Mix VAZYME P412 and the primer pair LYQ3/LYQ4 (Simon et al. 1994; 5'-CCTGGATCTTTA-ATTGGAGA-3' / 5'-GGTAAAATTAAAATATAAACTTC-3'). Sanger sequencing was carried out using an ABI 3730XL sequencer.

The COI sequences of other bombycids were extracted from corresponding complete mitochondrial genomes, including *Rotunda rotundapex* (Miyata & Kishida, 1990) (Park et al. 2020: NC045528), *Bombyx huttoni* Westwood, 1847 (Peng et al. 2015: KP216766), *Bombyx mandarina* Moore, 1872 (Kim et al. 2022: OK358669), *Bombyx mori* (Linnaeus, 1758) (Zhang et al. 2022: MW158386), *R. menciana* (Kim et al. 2014: KJ647172) and *Ocinara albicollis* (Walker, 1862) (Deng et al. 2022: OM912596), as well as the outgroup *Saturnia pyretorum* Westwood, 1847 (Saturniidae) (Jiang et al. 2009: FJ685653). A partial COI sequence of *R. diaphana* was also available (Lin et al. 2019: MH817453) and used in our analyses.

The different sequences were aligned with MAFFT V7 using default parameters (Katoh et al. 2019). The ML [maximum-likelihood] tree was generated with IQ-TREE 2 (Minh et al. 2020), running with the best-fitting model TIM2 + F + G4 (Kalyaanamoorthy et al. 2017) and ultrafast bootstrapping (Hoang et al. 2018).

In this paper, we use the terminology of Liu (2023) for the description of preimaginal stages, that of Zwick (2009) for genitalia structures, and we follow Comstock (1918: 332) and Kristensen (2003) for wing venation. The following abbreviations are used: $T_{1–3}$ = 1st–3rd thoracic segments; $A_{1–10}$ = 1st–10th abdominal segments; R4 = 4th radius; M3 = 3rd median vein; CuA1 = 1st anterior cubitus; 1A = 1st anal vein.

Results

Rondotia melanoleuca Cao & Liu, sp. nov.

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Holotype [HT]. Imago, 1 ♂ ([unique identifier: A23072701]: China, Dafengding, Leshan, Sichuan Province, ca. 1500 m (Fig. 1A, B). A red label "HOLOTYPE" will be added, and the holotype will be deposited at the Institute of Zoology, Chinese Academy of Sciences, Beijing.

Paratypes [PTs]. Imagines, 4 ♂♂ [unique identifiers: A23072702–A23072705] and 4 ♀♀ (unique identifiers: A23072706–A23072709] (e.g., Fig. 1C–H), same data as for HT. All are preserved in the collection of the senior author (Beijing) with blue paratype labels.

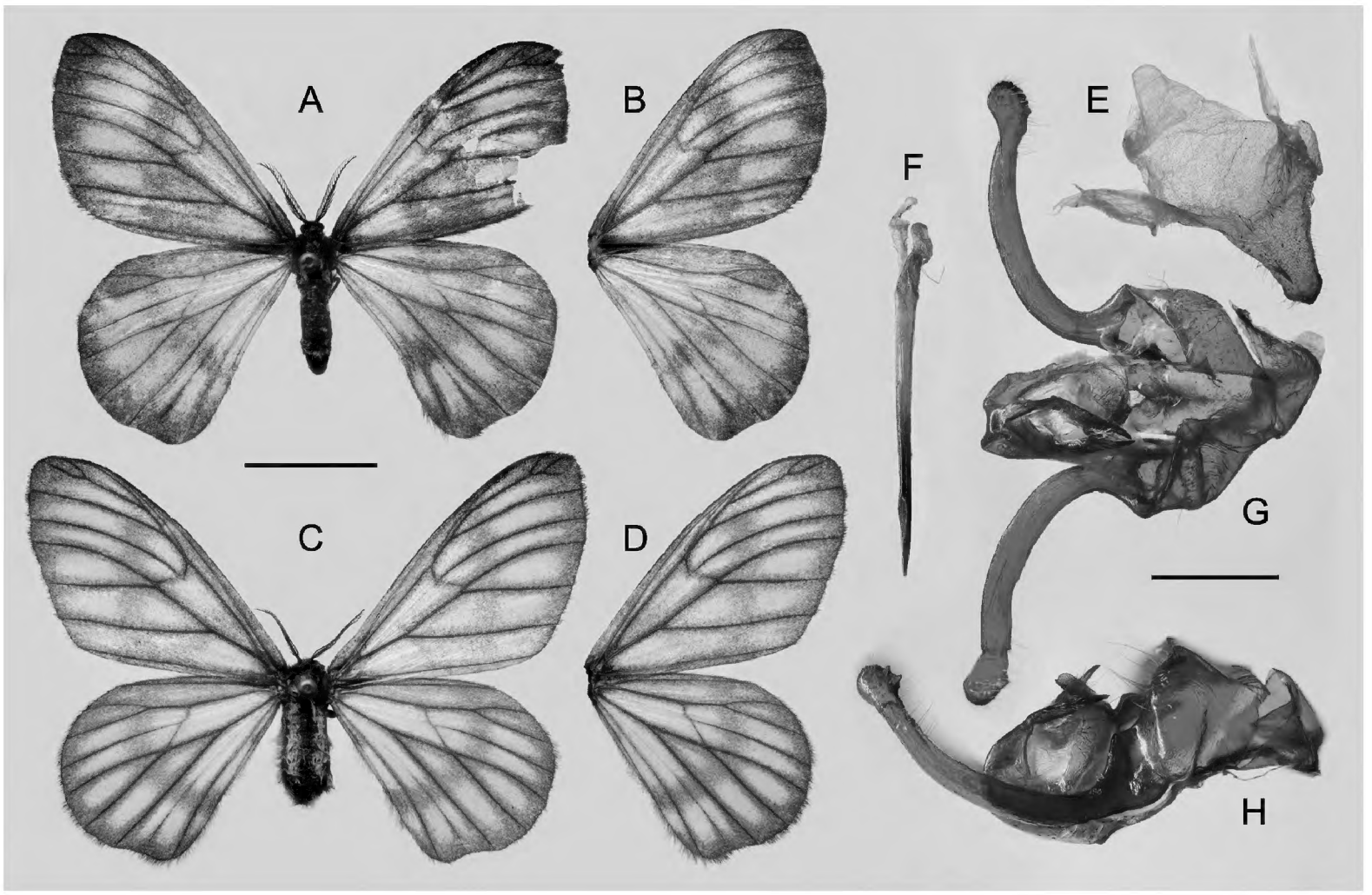

Figure 1. *R. melanoleuca* sp. nov. **A, B.** HT: ♂ **A.** Dorsal view; **B.** Fore- and hindwings, ventral view; **C, D.** PT [A23072706], ♀: **C.** Dorsal view; **D.** Fore- and hindwings, ventral view; **E–H.** PT [A23072704], ♂: **E.** Sternum A_8, dorsal view; **F.** Phallus, lateral view; **G, H.** Main genital sclerites of $A_{9–10}$; **G.** Posterior view; **H.** Lateral view. Scale bar (left) for **A–D:** 1 cm; scale bar (right) for **E–H:** 1 mm.

Descriptions. Mature Larvae (Fig. 2E, F):

Morphology. Head capsule is dark gray and sparse yellow powder is visible in some individuals, with yellowish adfrontal area, and labrum and basal antacoriae a pale yellow. The integumentary color of T_1–A_{10} is mostly vivid yellow, transversely decorated with black, dotted stripes. In lateral view, T_2 is the most swollen area dorsally. Legs $T_{1–3}$ are speckled with black on the lateral regions, while prolegs $A_{3–6}$ have a yellow ground color similar to the former. Caudal horn on middorsal A_8 is black and strongly developed.

Biology. A total of ten mature caterpillars were collected from a *Morus* sp. (Moraceae) in the wild at the type locality, on 02.v.2022. They were sent to Cao in Beijing and reared successfully in captivity on *Morus alba*. Liu had observed an individual on *Camptotheca acuminata* (Nyssaceae) at Fugong County, Yunnan, 1221 m, on 28.v.2023. After being brought back for indoor observations, it did not continue to feed on this plant or accept mulberry, and it ultimately starved to death. The caudal horn usually points to the posterosuperior side at rest, but it will swing back and forth (from anterior to posterior sides, with unstable frequency and speed) when frightened and crawling. After feeding ended, mature larvae always left their host plants to pupate in a cocoon. The larvae of HT and PTs span their cocoons ca. 05–09.v.2022.

Pupae (Fig. 2G, H):

Morphology of male and female pupae. The exterior shell is translucent after sclerotization is completed, showing yellowish tissues inside. Melanin is primarily pigmenting the spiracles $A_{2–8}$ of

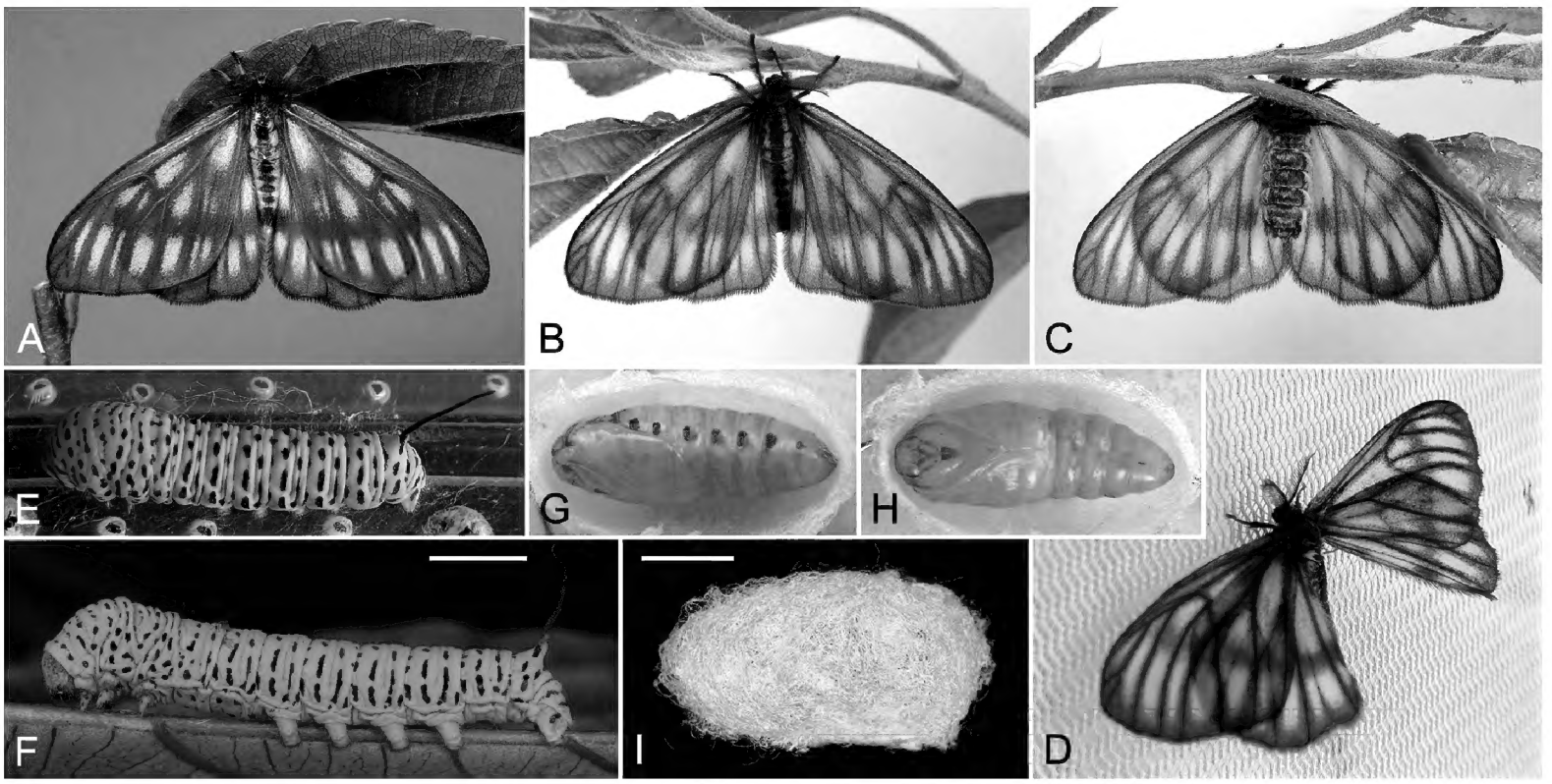

Figure 2. *R. melanoleuca* sp. nov. **A–E, G–I**. Specimens from the type locality; **F.** Specimen from Fugong County, Yunnan. **A.** Adult ♂, newly emerged, dorsal view, front light photography; **B, C.** Adult ♀, newly emerged, surround light photography: **B.** Dorsal view; **C.** Ventral view; **D.** Adult ♂, natural resting condition, dorsolateral view; **E.** Mature larva, dorsolateral view; **F.** Mature larva, lateral view; **G, H.** Pupa ♀, within an opened cocoon: **G.** Lateral view; **H.** Ventral view; **I.** Cocoon, lateral view. Scale bars: 5 mm.

the pupal exuviae, then distributed internally around the spiracle T_1, and also the areas of middorsal $A_{1–7}$ and dorsolateral $T_{1–3}$. The subterminal costal margins of forewings come in contact medioventrally; antennae, maxillae and legs $T_{1–3}$ are clearly visible.

Biology. During development of the adult in the pupa, the proximal regions of antennae, of the lateral areas of compound eyes, off the tarsi of legs T_1 and of the tip of A_{10} become pigmented blackish earlier than other structures.

Cocoons (Fig. 2G–I):

Morphology. White ellipsoid in general, about twice as long as wide, with the pupa nested inside along its longitudinal axis. The outer fibers form a wrinkled, irregular layer, that appears as a loose and perforated filament-layer in horizontal section. The innermost [pupal chamber] surface of the cocoon is regular and relatively smooth.

Imagines (Figs 1, 2A–D):

Morphology of males. Head largely covered by black scales, but the frontoclypeal area is off-white, with black compound eyes and bipectinate antennae. The labial palpus is but vestigial and very minute. The white scales are distributed on the subdorsal areas $T_{2–}A_8$, the subventral areas $A_{2–8}$ and the tip of A_9. The abdominal venter is grayish, while other areas of the whole body bear black scales; especially on T_1 and tegulae, these scales are longish and hair-like. Legs $T_{1–3}$ are also darkly colored, and the longest hair-like scales are located on the lateral margins of tibiae and basitarsi of forelegs. Both fore- and hindwings are semitransparent, the lengths are ca. 2.2 cm (from the wing base to the tip of R4, HT) and ca. 1.8 cm, (from the wing base to the tip of CuA1, HT), respective-

ly. Viewed from both dorsal and ventral sides, each wing-vein is located within a gray-black scaly stripe, such bands likewise decorated on the postbasal (only forewings), median (all wings), marginal (all wings, and here including costa-apex-termen-inners) areas, while other regions have a light cover with white scales. The termen of the hindwing is slightly concave around the tip of 1A.

The sternum A_8 is caudally digitiform and directed toward the posterior side, carrying dorsolateral bristles. Amongst the genital structures, tegumen and vinculum are fused and form a narrowly elliptic ring, with the vinculum extending into a very short saccus. The uncus is deeply bilobed, laterally sclerotized and medially membranous, its wedge-shaped terminal is inferolaterally setose. A pair of cambered sclerites derived from the gnathos is strongly developed, linked with the uncus by lateral membrane. The narrow and crescent valva is expanding terminally which ended with a rounded and smooth dorsodistal margin, each cucullus has serrated inferior margin with two or three identifiable pointed teeth bearing medioventral corona. The juxta is V-shaped and slightly protruding. The phallus is thin, long and straight, with a pointed tip that has a slightly helical opening; the caecum penis is well developed.

Morphology of females. Color pattern is nearly identical to males,but females are usually larger in size than males. Visually, wings appear narrower, longer and paler than in males, with lengths of forewing ca. 2.4 cm (from the wing base to the tip of R4, PT [A23072706]) and hindwing ca. 1.9 cm, (from the wing base to the tip of CuA1, PT [A23072706]). Prior to oviposition, $A_{2–7}$ medioventrally covered by many long, black scales.

Biology. Adults HT and PTs emerged from cocoons between 14 and 18.v.2022. In resting position, moths usually hold their forewings with their costal margins horizontal (perpendicular to the body), while the anal angles of the hindwings are widely separated from each other (Fig 2D). There was no successful pairing in captivity, but adults were found to be active during the day. Unfertile females nonetheless laid eggs, covering them with black scales from their abdominal midventral areas. It is noteworthy that the dorsal epicuticle of the membranous areas of wings appeared slightly iridescent while moths were alive, which was difficult to observe in dead, dry specimens.

Distribution. The specimens in this study are all from Leshan (Sichuan) and Fugong (Yunnan), but the new species may be more widely distributed in montane broadleaf forests in both provinces. However, due to the presence of similar environments, we presume the species may also be found in the northern Indochina Peninsula, e.g., in Myanmar, Laos, and Vietnam.

Diagnosis. Adults of *R. melanoleuca* sp. nov. have wings with distinct white patches placed between black vertical stripes and black wing-veins, which enables easy distinction from all other bombycids without dissection of genital structures. Wang et al. (2015) provided a diagnosis for *R. menciana*, *R. diaphana* and *R. lineata*, in which they noted "forewing with outer margin concave below apex and a projection at the end of M3 [...] uncus divided into three lobes; valval apex broader with a large and a small tooth". In contrast, the new species has a straighter forewing termen, a biforked uncus, and a rounded dorsodistal edge of the valva.

Etymology. This feminine adjective in modern Latin means "black and white"; interestingly, the Sichuanese giant panda carries the same specific name.

Molecular phylogeny. The phylogenetic tree (Fig. 3) reveals that *R. melanoleuca* sp. nov. is sister to *R. menciana* + *R. diaphana*, with a high support value of 99%. The generic relationships revealed by the topology obtained are in agreement with Wang et al. (2018) and Deng et al. (2022).

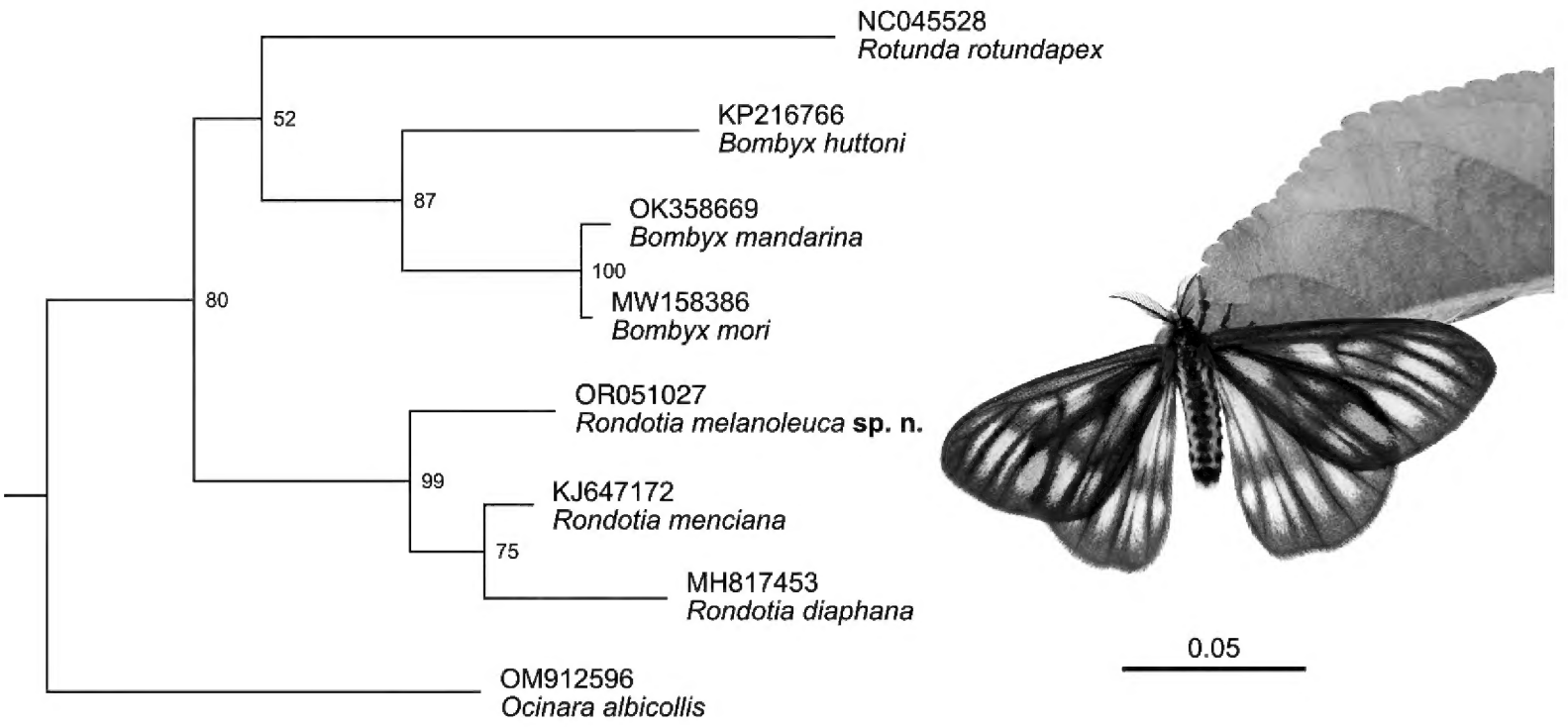

Figure 3. ML tree of the family Bombycidae (part) based on COI dataset, the outgroup (Saturniidae) is not shown. The scale bar indicates average substitutions per site. The nodal support is indicated by ultrafast bootstrap values.

Discussion and conclusion

This study introduces a very distinct new species with many unique phenotypic characteristics. The wing pattern in particular is completely different from the hypothetical ground plan of all other bombycines. However, we observe that the general morphology of the mature larva of *R. melanoleuca* sp. nov. is relatively similar to that of other known species of *Rondotia*, even though differences can be highlighted: *R. menciana* and *R. diaphana* (Anonymous 2020) mature larvae are whitish, covered with a distinct white powder, and spin yellow cocoons attached to leaves of their host plants.

The results of our phylogenetic analysis based on sequences of the COI gene have revealed that *R. melanoleuca* sp. nov. is sister to a clade formed by *R. menciana* and *R. diaphana* (see Fig. 3). Considering the very high morphological similarity of *R. lineata* with these two species, it can be assumed that the new species is sister to all other *Rondotia* species. This assumption agrees with the morphological uniqueness of *R. melanoleuca* sp. nov. in comparison to the relative uniformity of all other species known to date in the genus. Interestingly, the new species looks superficially like the butterfly genus *Aporia* Hübner, 1819 (Pieridae), and future ecological studies would be needed to clarify if a mimetic relationship exists between *R. melanoleuca* sp. nov. and other Lepidoptera species.

Acknowledgments

We are grateful to Andreas Zwick (CSIRO, Canberra) for providing considerable advice that helped improve the scientific quality of this article, as well as the comments from an anonymous reviewer. We thank Rodolphe Rougerie (MNHN, Paris) for editing the manuscript, Fei Lu (Southwest Forestry University, Kunming) and Siyao Huang (LIB, Museum Koenig, Bonn) offered kind help and suggestions, respectively. A special thanks goes to the photographer Yongxiang Zhou (Kunming), who assisted in the photography of Fig. 1E–H.

References

Anonymous (2020) *Rondotia diaphana*. [Facebook.] https://m.facebook.com/story.php?story_fbid=pfbid0trHUB6Z9t78QmECcnbi6XTLLyaKvRWLpE19ii4Bt4SWMvuftDEkQVFMQEZMPkb7Ql&id=100229525102083&sfnsn=mo [Date of access: 13.VII.2023]

Bai XC, Lou LJ, Yang MJ, Lu HY, Gao YM, Zhou YQ (2001) Dynamic analysis on the population of natural racial group of trivoltinism mulberry white caterpillar, *Rondotia menciana* Moore. Canye Kexue 27(2): 145–148. [in Chinese with English abstract]

Comstock JH (1918) The Wings of Insects: An Exposition of the Uniform Terminology of the Wing-Veins of Insects and a Discussion of the More General Characteristics of the Wings of the Several Orders of Insects. The Comstock Publishing Company, New York, 430 pp. [10 pls.] https://doi.org/10.5962/bhl.title.54605

Conte A (1911) Essai de classification des Lépidoptères producteurs de soie, 7e Fascicule. A. Rey, Lyon, 90 pp. [15 pls.]

Deng M, Liao CQ, Chen Q, Huang GH,Wang X (2022) Phylogenetic relationships among Bombycinae (Lepidoptera, Bombycoidea, and Bombycidae) based on mitochondrial genomes. Archives of Insect Biochemistry and Physiology 111(1): e21889. https://doi.org/10.1002/arch.21889

Hampson GF (1893) The Fauna of British India, Including Ceylon and Burma. Moths (Vol. 1). Taylor & Francis, London, 527 pp.

Hoang DT, Chernomor O, von Haeseler, Minh BQ, Vinh LS (2018) UFBoot2: Improving the ultrafast bootstrap approximation. Molecular Biology and Evolution 35(2): 518–522. https://doi.org/10.1093/molbev/msx281

Jiang ST, Hong GY, Yu M, Li N, Yang Y, Liu YQ, Wei ZJ (2009) Characterization of the complete mitochondrial genome of the giant silkworm moth, *Eriogyna pyretorum* (Lepidoptera: Saturniidae). International Journal of Biological Sciences 5(4): 351–365. https://doi.org/10.7150/ijbs.5.351

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. Nature Methods 14: 587–589. https://doi.org/10.1038/nmeth.4285

Katoh K, Rozewicki J, Yamada KD (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics 20(4): 1160–1166. https://doi.org/10.1093/bib/bbx108

Kim MJ, Park JS, Kim H, Kim SR, Kim SW, Kim KY, Kwak W, Kim I (2022) Phylogeographic relationships among *Bombyx mandarina* (Lepidoptera: Bombycidae) populations and their relationships to *B. mori* inferred from mitochondrial genomes. Biology 11(1): 1–68. https://doi.org/10.3390/biology11010068

Kim MJ, Jun J, Kim I (2014) Complete mitochondrial genome of the mulberry white caterpillar *Rondotia menciana* (Lepidoptera: Bombycidae). Mitochondrial DNA Part A 27: 731–733. https://doi.org/10.3109/19401736.2014.913163

Kristensen NP (2003) Skeleton and muscles: adults. In: Kristensen NP (Ed.) Handbook of Zoology (Vol. IV: Part 36), Lepidoptera, Moths and Butterflies. Morphology, Physiology and Development (Vol. 2). Walter de Gruyter, Berlin, 39–131. https://doi.org/10.1515/9783110893724.39

Leech JH (1898) Lepidoptera Heterocera from Northern China, Japan, and Corea. Transactions of the Entomological Society of London 1898: 261–380. https://doi.org/10.5962/bhl.title.22195

Lin RJ, Braby MF, Hsu YF (2019) The Immature Stages, Biology, and Phylogenetic Relationships of *Rotunda rotundapex* (Lepidoptera: Bombycidae). Journal of Insect Science 19(2): 1–11. https://doi.org/10.1093/jisesa/iez025

Liu ZY (2023) An "American" silkmoth endemic to Himalayas, part I: life history and natural distribution of *Antheraea compta* Rothschild, 1899 (Lepidoptera, Saturniidae). Deutsche Entomologische Zeitschrift 70(2): 261–282. https://doi.org/10.3897/dez.70.102952

Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, Lanfear R (2020) IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. Molecular Biology and Evolution 37(5): 1530–1534. https://doi.org/10.1093/molbev/msaa015

Moore F (1885) Description of a species of wild-mulberry silkworm, allied to *Bombyx*, from Chehkiang, N. China. The Annals and Magazine of Natural History, Including Zoology, Botany, and Geology 14: 491–492. https://doi.org/10.1080/00222938509459370

Park J, Xi H, Park J (2020) The complete mitochondrial genome of *Rotunda rotundapex* (Miyata & Kishida, 1990) (Lepidoptera: Bombycidae). Mitochondrial DNA Part B 5(1): 355–357. https://doi.org/10.1080/23802359.2019.1703589

Peng XY, Zhou P, Qiang Y, Qian ZQ (2015) Characterization of the complete mitochondrial genome of *Bombyx huttoni* (Lepidoptera: Bombycidae). Mitochondrial DNA Part A 27: 4112–4113. https://doi.org/10.3109/19401736.2014.1003868

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequence and a compilation of conserved polymerase chain reaction primers. Annals of the Entomological Society of America 87(6): 651–701. https://doi.org/10.1093/aesa/87.6.651

Tang SM, Wang HY, Wang XK, Liu LS, Pu YX, Wang XZ, Zhao QL, Cheng JL, Shen XJ (2018) Molecular cloning and identification of the hatching enzyme gene in *Rondotia menciana*. ScienceAsia 44 (2018): 234–240. https://doi.org/10.2306/scienceasia1513-1874.2018.44.234

Wang X, Chen ZM, Gu XS, Wang M, Huang GH, Zwick A (2018) Phylogenetic relationships among Bombycidae s.l. (Lepidoptera) based on analyses of complete mitochondrial genomes. Systematic Entomology 44(3): 490–498. https://doi.org/10.1111/syen.12337

Wang X, Wang M, Zolotuhin VV, Hirowatari T, Wu S, Huang GH (2015) The fauna of the family Bombycidae sensu lato (Insecta, Lepidoptera, Bombycoidea) from Mainland China, Taiwan and Hainan Islands. Zootaxa 3989(1): 1–138.

Wang X, Zheng L, Huang GH, Wang M, Kishida Y (2009) Discovery of *Rondotia diaphana*, comb. nov. (Lepidoptera, Bombycidae) from China. Tinea 21(1): 23–25.

Xu M, Zhang H, Zhu XF, Yan SJ, Chen J, Wang PS (1994) Studies on biological characters and control of mulberry white caterpillar, *Rondotia menciana* Moore. Canye Kexue 20(3): 136–140. [in Chinese with English abstract]

Zhang GZ, Zhang YL, Wei W, Li YP, Liu YQ, Bi LH, Lu C (2022) Mitochondrial genome architecture and evolutionary origin of the Yao silkworm, a living fossil of the domestic silkworm *Bombyx mori* (Lepidoptera: Bombycidae). Journal of Insect Science 22(2): 1–7. https://doi.org/10.1093/jisesa/ieac014

Zolotuhin VV, Witt TJ (2009) The Bombycidae of Vietnam. Entomofauna 16(Supplement): 231–272.

Zwick A (2009) The principal structure of male genital sclerites and muscles of bombycoid moths, with special reference to Anthelidae (Lepidoptera: Bombycoidea). Arthropod Structure & Development 38(2009): 147–161. https://doi.org/10.1016/j.asd.2008.07.006